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Discovery of Novel TAM Family Kinase Inhibitors

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Introduction

- TAM family (Tyro3, Axl, and Mer) of receptor tyrosine kinases mediated signaling is involved in cell survival, proliferation, migration and adhesion, vascular smooth muscle homeostasis, platelet function and erythropoiesis, and regulation of inflammatory cytokine release. These RTKs are frequently co-expressed in vascular, reproductive, nervous, and immune system in adults.
- TAM family kinases can be activated by vitamin K-dependent ligands Gas6 and Protein S. Tyro3, Axl, and Mer also display ectopic or over-expression in numerous cancers, including myeloid and melanoma, lymphoblastic leukemias, breast, lung, colon, liver, gastric, kidney, ovarian, uterine, and brain cancers.
- Therefore, the TAM family represent another class of kinase targets for development of cancer therapy.
- In this study, we report the discovery of a new class of potent inhibitors of TAM family kinases. These new compounds have demonstrated in vitro potency in nM range against the TAM family kinases and good selectivity against a selected kinase panel. The compounds can dramatically inhibit the phosphorylation of Akt at Ser-473 in cancer cell lines in a dose dependent manner and effectively inhibit the colony formation of cancer cells. In thymidine incorporation assay, many compounds showed inhibition of cell proliferation at sub-micromolar concentrations. Furthermore, many compounds have proven to have the desirable pharmaceutical properties. The most promising compounds have been selected for in vivo proof-of-concept studies. The data generated so far indicates that these new inhibitors of TAM family kinases represent a new approach for cancer therapy.

Target Validation

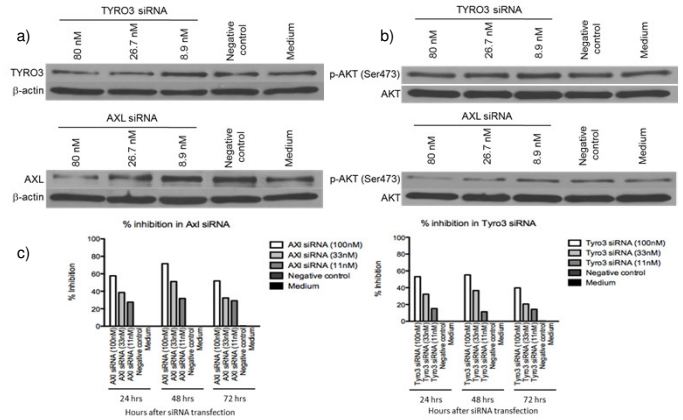

Figure 1. Effect of Tyro3 and Axl siRNA knockdown in A549 cells on: a) protein expression of Tyro3 and Axl; b) p-Akt (Ser-473) level; and c) inhibition of cell proliferation determined by thymidine incorporation assay. A dose dependent inhibition of Tyro3 and Axl protein expression, p-Akt level and the cell proliferation by the respective siRNA was observed.

SAR Summary

- >100 analogues have been synthesized and SAR trend has emerged.
- The potency has been improved over 100-fold.
- The correlation of potency obtained between biochemical assay and cell-based inhibition of pAkt expression assay is reasonably good.

Table 1. SAR summary. The potency was assessed using the ^{33}P radiometric assay with recombinant target kinases. The cell-based assay was done using A549 cells and the pAkt level was determined using western blot method and quantified with Image Lab. The effect on proliferation inhibition was determined using ^{3}H-labeled thymidine incorporation assay with A549 cells.

Code	AXL	TYRO3	MER	pAkt Inhibition	Proliferation
	IC_{50} (nM)			IC_{50} (μM)	IC_{50} (μM)
SLC-450	0.3	0.2	76.4	1.08	9.25
SLC-391	1.2	67.7	63.5	0.29	3.64
SLC-464	1.7	5.4	308.5	0.77	10.97
SLC-405	1.8	3.0	41.1	1.90	6.22
SLC-410	2.1	6.7	100.9	2.99	TBD
SLC-466	2.9	2.0	132.7	1.80	2.84
SLC-403	3.1	16.3	110.3	0.75	2.42
SLC-463	3.6	20.9	27.3	0.82	TBD
SLC-370	3.9	3.6	14.9	0.21	0.39
SLC-389	4.2	15.5	85.8	TBD	TBD
SLC-385	6.8	25.4	209.8	TBD	TBD
SLC-357	7.0	9.9	21.9	0.96	1.06
SLC-452	9.0	37.1	123.6	0.60	1.18
SLC-367	9.9	20.2	219.5	1.16	2.31
SLC-374	10.0	16.4	353.7	1.45	1.02
SLC-350	10.4	7.3	68.0	TBD	TBD
SLC-398	19.5	27.5	295.5	2.69	TBD
SLC-363	19.9	68.9	33.9	TBD	1.82
SLC-392	23.8	127.2	81.2	TBD	TBD

Anti-cancer Properties

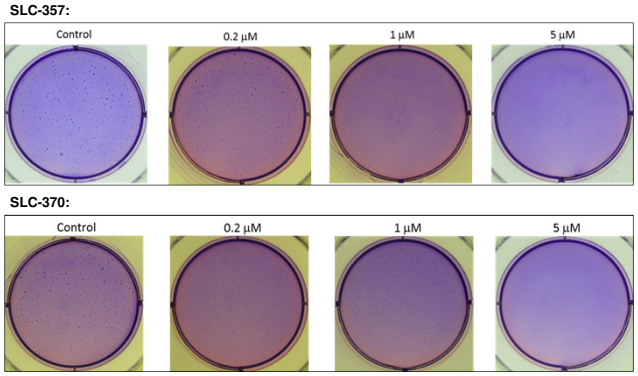

Figure 2. Inhibition of colony formation of A549 cells by compounds. Both compounds have effectively reduced the colony formation even at 0.2 μM. At 5 μM, The formation of colonies was completely inhibited.

Selectivity Profiles

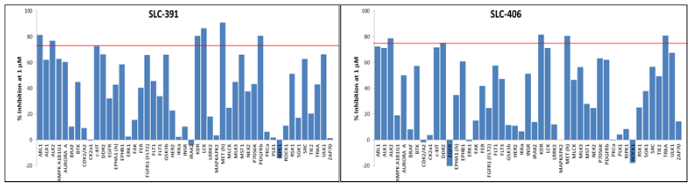

Figure 3. Selectivity profiles of selected compounds. These compounds are selective against TAM family kinases. Among the 47 kinases tested, only very few off-target kinases were inhibited to >75% at 1 μM.

ADME Profiles

Table 2. Summary of ADME profiles of selected compounds. Many of these compounds have desirable ADME profiles: reasonable solubility and metabolic stability in HLM and MLM, highly permeable and desirable CYP inhibition profiles. Currently, a set of selected compounds is in PK evaluation to identify candidates for proof of concept studies.

Code	Solubility	HLM	MLM	PAMPA	3A4	2D6	2C9	2C19
	(μM)	(% Remain., 30')		($LogP_{app}$)	(% Inhibition at 10 μM)			
SLC-350	174.81	91	74	-8.77	20	11	20	-5
SLC-357	4.28	100	98	-9.13	59	5	93	40
SLC-363	8.55	109	103	-9.35	6	-4	35	3
SLC-367	64.06	82	65	-9.61	9	7	13	0
SLC-370	3.89	70	51	-8.70	50	0	88	26
SLC-374	7.10	79	71	-8.96	-3	16	13	5
SLC-385	194.56	113	66	-9.55	-5	14	10	5
SLC-389	192.56	99	101	-9.37	-1	22	14	11
SLC-391	200.64	98	110	-9.37	22	9	9	-3
SLC-392	0.77	17	3	-5.66	79	33	38	55
SLC-398	169.15	79	77	-8.96	0	11	2	3
SLC-403	141.86	62	48	-9.38	37	6	21	3
SLC-405	7.55	47	16	-5.84	67	2	76	29
SLC-410	178.75	81	71	-9.29	5	5	8	0
SLC-450	140.77	102	60	-6.32	17	-2	46	5
SLC-452	180.45	81	71	-9.53	19	5	18	3
SLC-463	0.18	41	28	-5.54	38	7	0	-3
SLC-464	0.24	72	57	-5.08	8	15	0	3
SLC-466	3.55	85	72	-6.49	7	24	1	0

Summary

- A novel and potent series of TAM family kinase inhibitors was discovered.
- The potency has been increased to sub-nanomolar range.
- Many compounds have demonstrated sub-micromolar potency in inhibiting pAkt levels in cancer cells.
- These compounds also effectively inhibited cell proliferation and colony formation of cancer cells.
- Many compounds possess the desirable properties for further studies.
- Several compounds have been selected for POC studies.
- Targeting TAM family kinases may provide effective cancer therapy.